A Paler Shade of White: Multispectral Tissue Classification of Blockface Images During Human Brain Cryosectioning

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Introduction. A significant roadblock between the acquisition of functional data and its anatomical superposition is the absence of an accepted mapping between the classical postmortem histologies and the modern *in vivo* magnetic resonance imaging (MRI) anatomies. As part of an on–going effort to bridge this gap, we investigated the use of variable discrete wavelength imaging of the blockface of specimens undergoing cryosection in the hope of finding tissue based signals that could link histological data to ante- and post- mortem MRIs.

Methods. A high resolution camera with a dynamic liquid crystal filter was used to obtain a series of images of a single slice (on the block face) of a human head during the process of cryosectioning. Data were acquired at 5nm increments from 450nm to 1000nm and the spectra of several selected regions were computed. The data were also examined by a neuroanatomist to determine the relative clarity of fine structure at the various wavelengths. *Results.* Visual examination revealed anatomical features in the gray matter (Figures 1 and 2) whose presence became detectable only at certain wavelengths. At shorter wavelengths, the gray matter could easily be segregated into an outer higher intensity layer (presumably associated with the outer two cortical layers) whereas at longer wavelengths, this feature disappeared, but a fine band of lower intensity (coincident with the putative layer six) became visible. Spectral analysis (combined with multispectral classification) confirmed this observation, and further suggested that there were image characteristics within the white matter that varied over imaging wavelength and fiber orientation. For instance, fiber bundles oriented parallel to the plane of section (the internal capsule and corpus callosum) showed differing spectra from each other as well as from fibers oriented perpendicularly to the cutting plane (the cingulum). Neither the grey matter banding nor the white matter differences were observable to the naked eye or in 24 bit standard RGB color blockface images.

Conclusions. We conclude that multispectral imaging reveals detail otherwise unobservable in cryosection blockface digital imaging. These signals could be used to differentiate both within and between tissue classes, making this data source a useful tool for the regional parcellation of brain tissue. With the obvious extension to digitized histology, this technology could serve as a link between the high resolution cytoarchitectonic features observable under the microscope and the lower resolution of ante- and post- mortem MR imaging.

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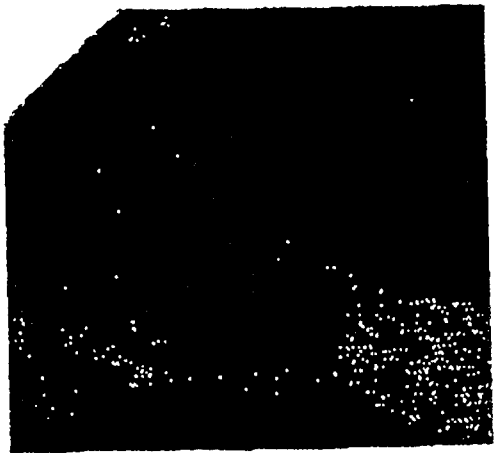

Figure 1: Appearance of cortex at 450nm. Note the visibility of the outer layers of the cortex.

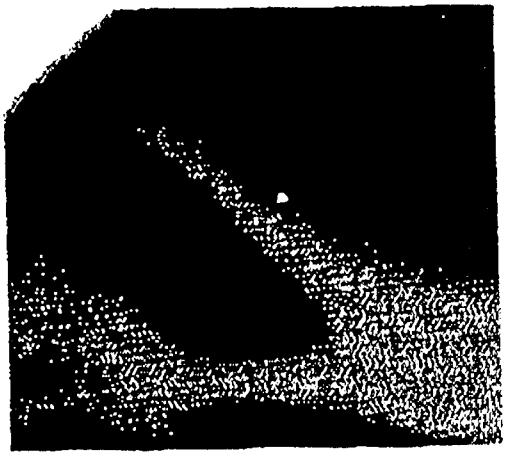

Figure 2: Appearance of cortex at 700nm. Note the loss of visibility of the outer layers of the cortex.